

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
17 January 2002 (17.01.2002)

PCT

(10) International Publication Number
WO 02/03855 A1

- (51) International Patent Classification⁷: **A61B 5/00**
- (21) International Application Number: **PCT/GB01/03078**
- (22) International Filing Date: **9 July 2001 (09.07.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
0016841.9 **7 July 2000 (07.07.2000)** **GB**
- (71) Applicant and
(72) Inventor: **STANLEY, Christopher, John** [GB/GB];
Harradine House, Church Street, Woodhurst, Huntingdon,
Cambridgeshire PE17 3BN (GB).
- (72) Inventor; and
(75) Inventor/Applicant (for US only): **WEBER, Anders**
[DK/DK]; Stormgardsvej 9, Skuldelev, DK-4050 Skibby
(DK).
- (74) Agent: **BOULT WADE TENNANT**; Verulam Gardens,
70 Gray's Inn Road, London WC1X 8BT (GB).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/03855 A1

(54) Title: OPTICAL DEVICE FOR MEASUREMENT OF ANALYTES IN TEARS

(57) Abstract: A device for use in the measurement or monitoring of analytes in lachrymal fluid using optical techniques and methods of measuring analytes using the device.

- 1 -

Optical device for measurement of analytes in tears

The present invention relates to a device for use in the measurement or monitoring of analytes in lachrymal fluid using optical techniques and to an analyte monitoring system using this device. The device is particularly suited for use in situations in which analyte levels must be closely monitored, for example with drugs that must be maintained within a narrow therapeutic window and where analyte measurements must be taken repeatedly, such as in long term diabetes.

In the management of diabetes, the regular measurement of glucose in the blood is essential in order to ensure correct insulin dosing. Furthermore, it has been demonstrated that in the long term care of the diabetic patient better control of blood glucose levels can delay, if not prevent, the onset of retinopathy and other degenerative diseases often associated with diabetes. Thus there is a need for reliable and accurate self-monitoring of blood glucose levels by diabetic patients.

Currently, blood glucose is monitored by diabetic patients with the use of commercially available colorimetric test strips or electrochemical biosensors (e.g. enzyme electrodes), both of which require the regular use of a lancet-type instrument to withdraw a suitable amount of blood each time a measurement is made. On average, the majority of diabetic patients would use such instruments to take a measurement of blood glucose approximately twice a day. However, the National Institutes of Health in the United States recently recommended that blood glucose testing should be carried out at least four times a day, a recommendation that has been endorsed by the American Diabetes Association. This increase in the frequency of blood glucose testing imposes a considerable burden

- 2 -

on the diabetic patient, both in terms of financial cost and in terms of pain and discomfort, particularly in the long term diabetic who has to make regular use of a lancet to draw blood from the fingertips. Thus, there is clearly a need for a better long term glucose monitoring system that does not involve drawing blood from the patient.

There have been a number of recent proposals for non-invasive or minimally invasive glucose measurement techniques. In particular, various approaches have concentrated on the use of the eye as a readily accessible compartment of the body in which to monitor glucose levels. European patent application number EP-A-792619 describes the use of infra-red spectroscopy to quantitate glucose in the exposed capillaries of the retina. This technique is obviously minimally invasive but suffers from a lack of sensitivity due to the fact that the infra-red spectrum of glucose in the capillaries of the retina is so similar to that of the surrounding tissue that in practical terms it is virtually impossible to resolve the two spectra.

Alternative approaches have exploited the finding that the aqueous humour of the eyeball itself contains glucose at a concentration which correlates with the level of glucose in serum. For example, European patent application number EP-A-603658 describes a technique for determination of the glucose concentration in the aqueous humour of the eye using polarimetry. Again this technique is minimally invasive but has proven to be of limited value because of its low sensitivity.

Improved techniques for the measurement of glucose in the eye have exploited recent observations that the concentration of a particular analyte in tears (lachrymal fluid) correlates with the concentration of that same analyte in blood. Of

- 3 -

particular relevance to diabetics, it is known that lachrymal fluid contains glucose and that the level of glucose changes in response to feeding. For example, SU-A-1534406 describes a method of diagnosing latent
5 diabetes mellitus which involves administering glucose orally and subsequently monitoring the concentration of glucose in tears, a study which indicates that glucose appears in lachrymal fluid after oral ingestion. This observation is especially relevant
10 when considering the self-monitoring of glucose by diabetic patients where it is essential that the glucose measurement be sensitive to dietary intake of sugars. Lachrymal fluid is thus a good target for non-invasive glucose measurement.

15 Current techniques for measuring glucose in lachrymal fluid include the use of basic colorimetric test strips which are wetted by tears, either directly or with the use of an absorbent wick, and then inspected for a colour change (described in EP-A-
20 236023 and US-A-5352411). Alternatively, Japanese Patent Application No. JP-A-05093723 describes a device constructed of a 'plastics material' which is fitted to the eyeball and is equipped with an electrochemical system using an enzyme based sensor
25 for measuring analytes, such as glucose or lactate, in the lachrymal fluid. The electrochemical sensor system carries an associated risk of the production of unwanted products as a result of enzymatic or electrochemical reactions in the sensor. Moreover the
30 actual device fitted to the eyeball is unwieldy as it must incorporate, at the very least, immobilised enzyme, a reaction electrode, an auxiliary electrode, a remote measurement transmitter and a battery.

WO 01/13783 describes the use of an ophthalmic
35 lens comprising a receptor moiety which can be used to determine the amount of an analyte in an ocular fluid. The ophthalmic lens can be a full size contact lens, a

- 4 -

permanently implanted lens of the intraocular, intracorneal or subconjunctival type or a shunt or implant in the cul de sac of the eye. Ophthalmic lenses can be corrective or they may not affect visual acuity, the lenses must also be accessible to light. The examples indicate that the lenses are illuminated through the pupil of the eye; no indication is given as to how a signal can be obtained from ophthalmic lenses that are accessed through other routes. Permanently implanted lenses are not ideal for a sensing application as the sensor components only have a limited lifetime.

The present invention provides a device which avoids the disadvantages of the prior art. Accordingly, in a first aspect the present invention provides a device for the detection or quantitative measurement of an analyte in lachrymal fluid, the device being locatable on the surface of the eyeball in contact with lachrymal fluid and incorporating an assay for said analyte, the readout of which assay is a detectable or measurable optical signal which can be interrogated remotely using optical means.

The device of the present invention has the advantages of reliability and accuracy of glucose measurement, safety, convenience and ease of use. With particular relevance to the long-term care of diabetics, the device has the additional advantage that glucose measurements can be taken as often as is necessary with no adverse effects on the patient. As glucose measurements can be taken more frequently, tighter control can be maintained over the level of glucose in the blood and the risk of developing conditions related to poorly regulated blood glucose, such as retinopathy and poor circulation, will be reduced.

The device of the present invention may be in the

- 5 -

form of a full size contact lens. In one embodiment, the device of the invention may be in the form of a full size contact lens which is optically correcting. In this embodiment the device would replace "normal" optically correcting contact lenses in, for example, diabetic patients who have a need for both regular self-administered glucose testing and for optical correction of poor eyesight. The device may be manufactured in the form of "disposable" contact lenses which are intended to be worn for a finite period of time, such as one day, and then disposed of.

In a preferred embodiment the device may be sized so as to permit location solely behind an eyelid of the user, preferably the lower eyelid, such that the device does not cover the iris or pupil and therefore does not have any effect on the vision of the wearer. A device located in this position is less invasive than a full size contact lens and will be better tolerated by patients. In this embodiment the device is preferably adapted (e.g. by virtue of its size and/or shape and/or surface coating) to be retained behind the eyelid, most preferably the lower eyelid, when the device is in use. In this "sub-eyelid" configuration the device will be in intimate contact with the membrane on the inside of the eyelid and will therefore remain hydrated at all times. The device may be interrogated externally through the tissue of the eyelid, for example using a fluorimeter device of the type described herein. Interrogation of the device may take place with the eye open or closed.

Since the "sub-eyelid" device does not cover the pupil there is no requirement for it to be transparent to visible light. In one embodiment the device may be coated with a reflective backing on the back-surface of the device, meaning the surface which is in contact with the surface of the eye when the device is in use. In a further embodiment the front surface of the

- 6 -

device, i.e. that which is in contact with the inside of the eyelid, may be coated with a tissue adhesive polymer.

5 The device may be constructed of any material known in the art to be suitable for the construction of contact lenses, the soft lens material is preferred since this is a hydrogel so good contact between lachrymal fluid and the assay components is assured. Alternatively the assay components may be bonded to or
10 located on the surface of a hard lens material.

Soft lens materials are permeable to low molecular weight analytes, such as glucose, which can thus enter the device by diffusion and interact with the reactive components which make up the assay for an
15 analyte. The reactive components of the assay may be uniformly incorporated throughout the structure of the device or alternatively may be localised to one area of the device in a discrete zone or spot. In one embodiment, the reactive components are incorporated
20 in a strip on the periphery of the device and where the device is in the form of a disc-shaped contact lens the reactive components can be incorporated into a circumferential strip on the periphery of the lens. The incorporation of the reactive components of an
25 assay into the structure of a contact lens does not affect visual acuity, or light transmission through the lens, to a significant extent.

The reactive components may be bound to the polymer matrix of the lens itself i.e. the hydrogel
30 acrylic based material from which the lens is made. In this case the reactive components of the assay can be linked to the monomers prior to polymerisation. Alternatively these components can be added to the monomer mix and then entrapped in the structure of the
35 lens following polymerisation. In this case it is advantageous to use high molecular weight components such as polymers to which the analyte analog component

- 7 -

of the assay (as will be defined below) is bound in order to minimise leakage by diffusion from the lens during use. A further configuration is a coated lens with another outer layer of hydrogel containing the reactive components, or with the reactive components bound directly to the outside surface of the lens after manufacture.

As an alternative to the contact lens format the device may also be constructed in the form of a fluid composition comprising a fluid matrix in which the reactive components of the assay are uniformly suspended. Thus, similar assay means to those employed in the contact lens device hereinbefore described can be delivered to the eye in fluid form, preferably using the familiar eye drop delivery system. The fluid containing the assay components will migrate in the eye, lodge behind the eyelid and equilibrate with the lachrymal fluid. In order to prevent rapid loss of the assay components from the eye the fluid matrix in which the reactive components are suspended may be viscous or may form a gel following migration behind the eyelid. This "fluid form" device is interrogated remotely using the same optical means as is used to interrogate the contact lens device.

The assay components may also be delivered to the eye in a suspended microcapsule format, wherein the reactive assay components are encapsulated within the microcapsules which are sufficiently small in diameter not to cause irritation to the eye. The microcapsules may be suspended in a tissue-safe sterile fluid carrier such as, for example, sterile isotonic saline. The outer surface of the microcapsules may be derivatised with specific binding agents or polymers that are known to bind to tissue, such as polyacrylic acid, to ensure that the microcapsules are retained at the site of application, or remain lodged behind the

- 8 -

eyelid, for extended periods. The microcapsule wall can remain intact throughout the lifetime of the sensor in the eye, thus preventing contact of the sensor chemistry components with the tissue structures of the eye, whilst allowing passage of analytes from the lachrymal fluid.

The sensor chemistry may also be embedded in microparticles constructed of biocompatible or biodegradable materials, for example polylactide-polyglycolide (PLGA), polyanhydride, polyhydroxybutyrate, polyacrylamide, agarose, cellulose or starch. The microparticles are preferably provided in the form of a suspension in a tissue-safe fluid carrier such as, for example, sterile isotonic saline. In this configuration the microparticles will be retained at the site of application on the inner surface of the eyelid or outer surface of the eyeball and if constructed of biodegradable polymers will be degraded at a controlled rate by hydrolysis or dissolution of the structural polymer. In the case of a fluid matrix or particulate sensor device the sensor chemistry components and associated materials will eventually be lost from the eye and will need to be replenished.

Fluid matrix-based devices, include those based on microparticles or microcapsules, which in use become located, in whole or part, behind an eyelid of the user may be interrogated through the eyelid, for example using a fluorimeter device of the type described herein.

A preferred assay for incorporation into the device comprises a binding assay, the readout of which is a detectable or measurable optical signal which can be interrogated remotely using optical means. The binding assay generating the optical signal should preferably be reversible so that a continuous monitoring of fluctuating levels of the analyte can be

- 9 -

achieved. This reversibility is a particular advantage of the use of a binding assay format in which the reactive components of the assay are not consumed. Binding assays are also preferred for use
5 in the device of the invention for reasons of safety as they cannot generate any unwanted products as could an enzymatic or electrochemical reaction.

Preferred binding assay configurations include a reversible competitive, reagent limited, binding
10 assay, the reactive components of which include an analyte analog and an analyte binding agent capable of reversibly binding both the analyte of interest and the analyte analog. The analyte of interest and the analyte analog compete for binding to the same binding
15 site on the analyte binding agent. Such competitive binding assay configurations are well known in the art of clinical diagnostics and are described, by way of example, in the Immunoassay Handbook, ed. David Wild, Macmillan Press 1994. Suitable binding agents for use
20 in the assay include antibodies or fragments thereof which contain an antigen binding site (e.g. Fab fragments), lectins e.g. concanavalin A, hormone receptors, drug receptors, enzymes, aptamers, nucleic acids or nucleic acid analogue structures that have
25 specific binding capability and molecularly-imprinted polymers based on acrylic and other plastics materials. Protein binding agents may be modified by, for example, polyethylene glycol derivatisation to increase stability and reduce irritancy and
30 allergenicity.

A further binding assay configuration suitable for use in the device is the two-site 'sandwich' or reagent excess assay. In this case two specific analyte binding agents bind to separate sites on the
35 analyte, which can be a protein or other analyte sufficiently large to have two epitopes. The sandwich assay configuration does not involve interaction

- 10 -

between the two binding agents incorporated into the contact lens unless an analyte is present. In contrast, the competitive assay format will already have formed a complex between the analyte analog and the binding agent in a pre-wetted contact lens. Thus when the analyte enters the contact lens it displaces the analyte analog from the binding agent and an equilibrium is reached quickly.

The competitive and sandwich assays can be reversible or non-reversible depending on the affinity constant of the binding agents. In the case of a very high affinity constant the assay is effectively non-reversible since dissociation of the complex will not take place within a useful measurement interval i.e. seconds to minutes. Thus, with a high affinity system the lens may be used as a threshold sensor useful for indicating (on a 'one-off' basis) an increase in the concentration of an analyte in lachrymal fluid.

Suitable optical signals which can be used as an assay readout in accordance with the invention include any optical signal which can be generated by a proximity assay, such as those generated by fluorescence energy transfer, fluorescence polarisation, fluorescence quenching, phosphorescence techniques, luminescence enhancement, luminescence quenching, diffraction or plasmon resonance, all of which are known *per se* in the art.

The most preferred embodiment of the device of the invention incorporates a competitive binding assay providing an optical readout based on the technique of fluorescence energy transfer. In this assay format the analyte analog is labelled with a first chromophore (hereinafter referred to as the donor chromophore) and the analyte binding agent is labelled with a second chromophore (hereinafter referred to as the acceptor chromophore). It is an essential feature of the assay that the fluorescence emission spectrum

- 11 -

of the donor chromophore overlaps with the absorption spectrum of the acceptor chromophore, such that when the donor and acceptor chromophores are brought into close proximity by the binding of the analyte analog to the analyte binding agent a proportion of the fluorescent signal emitted by the donor chromophore (following irradiation with incident radiation of a wavelength absorbed by the donor chromophore) will be absorbed by the proximal acceptor chromophore (a process known in the art as fluorescence energy transfer) with the result that a proportion of the fluorescent signal emitted by the donor chromophore is quenched and, in some instances, that the acceptor chromophore emits fluorescence. Fluorescence energy transfer will only occur when the donor and acceptor chromophores are brought into close proximity by the binding of analyte analog to analyte binding agent. Thus, in the presence of analyte, which competes with the analyte analog for binding to the analyte binding agent, the amount of quenching is reduced (resulting in an increase in the intensity of the fluorescent signal emitted by the donor chromophore or a fall in the intensity of the signal emitted by the acceptor chromophore) as the labelled analyte analog is displaced from binding to the analyte binding agent. The intensity of the fluorescent signal emitted by the donor chromophore thus correlates with the concentration of analyte in the lachrymal fluid bathing the device.

An additional advantageous feature of the fluorescence energy transfer assay format arises from the fact that any fluorescent signal emitted by the acceptor chromophore following excitation with a beam of incident radiation at a wavelength within the absorption spectrum of the acceptor chromophore (but not within the absorption spectrum of the donor chromophore) is unaffected by the fluorescence energy

- 12 -

transfer process. It is therefore possible to use the intensity of the fluorescent signal emitted by the acceptor chromophore as an internal reference signal, for example in continuous calibration of the device.

5 Competitive binding assays using the fluorescence energy transfer technique which are capable of being adapted for use in the device of the invention are known in the art. For example, US Patent No. 3,996,345 describes immunoassays employing antibodies and fluorescence energy transfer between a fluorescer-
10 quencher chromophoric pair. Meadows and Schultz (Anal. Chim. Acta. (1993) 280: pp21-30) describe a homogeneous assay method for the measurement of glucose based on fluorescence energy transfer between
15 a labelled glucose analog (FITC labelled dextran) and a labelled glucose binding agent (rhodamine labelled concanavalin A). In all of these configurations the acceptor and donor chromophores/quenchers can be
20 linked to either the binding agent, the analyte analog or, in the case of sandwich assays, to either binding agent.

 As an alternative to intensity based fluorescence resonance energy transfer, phase modulation
 fluorimetry can be used (Lakowicz and Maliwal,
25 Analytical Chimica Acta 271, (1993), 155-164). This involves the measurement of donor decay times, typically a donor fluorophore can be linked to a specific binding agent and a decreased fluorescence decay time is observed upon binding of acceptor
30 labelled analytes. The advantage of lifetime measurements is that the signal is not greatly affected by changes in fluorescence intensity or by absorption in the surrounding tissue of the eye.

 A further alternative to fluorescence energy
35 transfer is the fluorescence quenching technique. In this case a compound with fluorescence quenching capability is used instead of the specific acceptor

- 13 -

chromophore and the optical signal in a competitive binding assay will increase with increasing analyte. An example of a powerful and non-specific fluorescence quencher is given by Tyagi et al. Nature Biotechnology
5 (1998) 18: p49.

The device of the invention can be adapted for the detection or quantitative measurement of any analyte present in lachrymal fluid. Preferred analytes include glucose (in connection with the long-
10 term monitoring of diabetics), urea (in connection with kidney disease or dysfunction), lactate (in connection with assessment of muscle performance in sports medicine), ions such as sodium, calcium or potassium and therapeutic drugs whose concentration in
15 the blood must be closely monitored, such as, for example, digoxin, theophylline or immunosuppressant drugs. The above analytes are listed by way of example only and it is to be understood that the precise nature of the analyte to be measured is not
20 material to the invention.

The device is interrogated remotely using optical means which supply an eye-safe level of incident radiation, no physical contact being required between the device and the optical means. When the device
25 incorporates a competitive, reagent limited, or a sandwich, reagent excess, binding assay employing the technique of fluorescent energy transfer, the optical means should supply a first beam of incident radiation at a wavelength within the absorption spectrum of the
30 donor chromophore and preferably a second beam of incident radiation at a wavelength within the absorption spectrum of the acceptor chromophore. In addition, the optical means should be capable of measuring optical signals generated in the device at
35 two different wavelengths; wavelength 1 within the emission spectrum of the donor chromophore (the signal

- 14 -

generated in connection with the measurement of
analyte) and wavelength 2 in the emission spectrum of
the acceptor chromophore (which could be the analyte
signal or the internal reference or calibration
5 signal).

Optical means suitable for use in remote
interrogation of the device of the invention include a
simple high-throughput fluorimeter comprising an
excitation light source such as, for example, a light-
10 emitting diode (blue, green or red > 1000 mCa), an
excitation light filter (dichroic filter), a
fluorescent light filter (dichroic or dye filter) and
a fluorescent light detector (PIN diode
configuration). A fluorimeter with these
15 characteristics may exhibit a sensitivity of between
picomolar to femtomolar fluorophore concentration.

A suitable fluorimeter set-up is shown in the
accompanying Figure 1 and described in the Examples
included herein. Briefly, the fluorimeter comprises a
20 light-emitting diode (1) providing an excitation light
beam which passes through a condenser (2) containing
an excitation filter (3) and is incident upon a
beamsplitter (4). Part of the excitatory beam is
thereby deflected into launching optics (5) and enters
25 an optical fibre (6). In use the optical fibre is
placed close to fluorescent sensor chemistry either in
a lens, or a device located behind the eyelid. A
portion of the optical signal emitted from the sensor
chemistry following excitation enters the optical
30 fibre (6) and is thereby conveyed into the fluorimeter
where it passes through a blocking filter (8) and is
measured by a signal detector diode (7). The
fluorimeter also contains a reference detector diode
(9) which provides a reference measurement of the
35 excitatory light emitted from the LED (1). The
fluorimeter is battery operated (typical power
consumption 150mA at 9v) and for convenience can be

- 15 -

constructed in the shape and dimensions of a pen,
watch or cellular phone.

5 The fluorimeter separately measures the following
parameters:

At wavelength 1 (donor chromophore)

Excitation light intensity, $I(1,0)$

Ambient light intensity, $I(1,1)$

10 Intensity of combined fluorescent and
ambient light, $I(1,2)$

At wavelength 2 (acceptor chromophore)

Excitation light intensity, $I(2,0)$

15 Ambient light intensity, $I(2,1)$

Intensity of combined fluorescent and
ambient light, $I(2,2)$

20 Measurements are taken by holding the fluorimeter
close to the sensing device in contact with the eye.
The signal therefore travels a short distance through
air or eyelid tissue from the sensing device to the
fluorimeter but it is generally not necessary to
correct for any attenuation of light from source to
25 detector as the same attenuation will apply for both
the excitatory and the emitted light. The final output
provided is the normalized ratio between the
fluorescent intensity from the two fluorophores,
defined by the following relation (Equation 1):

30

$$\frac{(I(1,2)-I(1,1))}{(I(2,2)-I(2,1))} \frac{I(2,0)}{I(1,0)} \quad (1)$$

35 In a second aspect the present invention provides
an analytical system for the detection or quantitative
measurement of an analyte in lachrymal fluid, which

- 16 -

analytical system comprises;

5 (i) a device for the detection or quantitative measurement of an analyte in lachrymal fluid, said device being locatable on the surface of the eyeball in contact with lachrymal fluid and said device incorporating an assay for said analyte, the readout of which assay is a detectable or measurable optical signal which can be
10 interrogated remotely using optical means.

(ii) optical means for interrogating the device of part (i).

15 In a third aspect the present invention provides a method of quantitatively measuring the concentration of an analyte in the lachrymal fluid of a patient, said method comprising the steps of;

20 (a) locating a device in accordance with the first aspect of the invention in contact with the eyeball of said patient;

(b) allowing the assay of said device to reach
25 thermodynamic equilibrium;

(c) interrogating the readout of said assay using optical means; and

30 (d) relating the measurement obtained in (c) to the concentration of analyte.

The final output from the optical means (e.g. the fluorimeter) as given by Equation 1 above is converted
35 to analyte concentration preferably by means of a computer using calibration data which can be obtained based on the principles set out below.

- 17 -

A calibration curve can be established empirically by measuring response versus analyte concentration for a physiologically relevant range of analyte concentrations. Preferably this takes place *in vitro* as part of the production of the sensor device. The calibration procedure can be simplified considerably by using the mathematical relation between response and analyte concentration in a competitive affinity sensor which is derived as follows:

The response of a competitive affinity sensor is governed by the reactions:



designating the dissociation of the complexes RC and RL, formed by the combination of analyte binding agent (R) with analyte (L) or analyte analog (C).

The corresponding dissociation equilibrium constants are:

$$K_1 = \frac{C_R C_C}{C_{RC}}$$

and,

$$K_2 = \frac{C_R C_L}{C_{RL}}$$

where C designates the number of moles of the species in the sensor divided by the sensor volume. Using this measure of concentration both immobilized species and species in solution are treated alike.

- 18 -

The mass balance equations are:

$$T_c = C_c + C_{RC}$$

5 for total analyte analog concentration and,

$$T_R = C_R + C_{RC} + C_{RL}$$

for total analyte binding agent concentration.

10

Using the expressions above, the relation between response and analyte concentration is derived:

$$15 \quad \frac{T_c - C_c}{C_c} K_1 = \frac{T_R - (T_c - C_c)}{1 + (C_L/K_2)} \quad (2)$$

By using this relation the amount of data necessary
20 for the calibration can be reduced to two key parameters: total analyte binding agent concentration and total analyte analog concentration. The calibration curve is thus determined by two points on the curve.

25 In a preferred embodiment of the method of the invention the device used is a device adapted such that at least a part of it is retained behind the eyelid of the patient when in use. Suitable devices include the sub-eyelid and fluid matrix devices
30 described herein. The readout of the assay may then be interrogated through the tissue of the eyelid.

The present invention will be further understood with reference to the following non-limiting Examples,
35 together with the accompanying Figures in which:

Figure 1 is a schematic diagram of the optical part of

- 19 -

a fibre optic fluorimeter.

Figure 2 is a schematic diagram of a driver/amplifier circuit used in conjunction with the optical part of the fibre optic fluorimeter.

Figure 3 is a schematic cross section of a human eye.

Figure 4 is an enlarged cross section through the eyelids and cornea illustrating the placement of a sub-eyelid device according to the invention in use.

Referring to the drawings, Figures 3 and 4 are schematic cross-sections of the human eye showing the spatial arrangement of the upper eyelid (20), lower eyelid (21) and cornea (22). The cul de sac of the eye is indicated (23). Figure 4 illustrates how a sub-eyelid device (24) is placed against the membrane lining the inner surface of the eyelid, such that it may be interrogated through the tissue of the eyelid using optical means held external to the eye. The device is shown positioned inside the lower eyelid. However, it may also be positioned inside the upper eyelid.

25

Example 1

A disposable soft contact lens (Johnson & Johnson, Acuvue, etafilcon A) of diameter 14.2 mm and correction -2.25 was removed from its packaging, blotted to remove excess storage fluid and placed in the normal position in the eye of a human subject. After wearing the lens for one hour to allow equilibration with the lachrymal fluid the lens was removed and replaced in the container in which the lens had been delivered from the manufacturer. Then 200 µl of extracting fluid (distilled water) was

- 20 -

pipetted onto the lens and the container was placed on a laboratory rocking table to ensure that the entire lens was in contact with the extracting fluid. The lens was incubated at room temperature for one hour to ensure equilibration of the glucose retained within the lens structure with the surrounding extracting fluid. A home glucose test system from Roche Diagnostics' Ltd, Lewes, UK (Glucotrend 2) was calibrated and a glucose measurement test strip was inserted into the reader. Then 20 μ l of the contact lens extracting fluid was pipetted onto the glucose measurement test trip and a reading was obtained according to the manufacturer's instructions. The concentration of glucose measured in the eluate from the lens by the Glucotrend 2 meter was 3.9 mmol/l

Example 2

The procedure of Example 1 was repeated, except that the human subject had ingested 100 g of food grade glucose dissolved in distilled water 65 minutes prior to placing the lens in the eye. The concentration of glucose measured by the Glucotrend 2 meter in the extracting fluid from the lens was 6.7 mmol/l.

Example 3

A disposable soft contact lens (Johnson & Johnson, Acuvue, etafilcon A) of diameter 14.2 mm and correction -2.25 was removed from its packaging, blotted to remove excess storage fluid and cut in half to create a device suitable for location behind the eyelid. The half lens device was then placed in the eye of a human subject and moved downwards so that the device was entirely covered by the lower eyelid. The device was not visible and did not project above the edge of the lower eyelid. The procedures of Examples

- 21 -

1 and 2 were repeated, except 100 μ l of extracting fluid was used. The concentration of glucose measured by the Glucotrend 2 meter in the extracting fluid from the device was 3.1 mmol/l. before and 5.5 mmol/l
5 measured 65 minutes after ingesting 100 g of glucose.

Example 4

A fibre optic spectrometer was assembled as follows:

The optical part of a fibre optic fluorimeter was
10 made from standard components on a micro bench. The setup, comprising a red LED as light source, lenses, dichroic beamsplitter and filters and detector diodes, was as shown in Figure 1. Briefly, the fluorimeter comprises a light-emitting diode (1) providing an
15 excitation light beam which passes through a condenser (2) containing an excitation filter (3) and is incident upon a beamsplitter (4). Part of the excitatory beam is thereby deflected into launching optics (5) and enters an optical fibre (6). In use
20 the optical fibre is placed close to fluorescent sensor chemistry either in a lens or a device located behind the eyelid. A portion of the optical signal emitted from the sensor chemistry following excitation enters the optical fibre (6) and is thereby conveyed
25 into the fluorimeter where it passes through a blocking filter (8) and is measured by a signal detector diode (7). The fluorimeter also contains a reference detector diode (9) which provides a reference measurement of the excitatory light emitted
30 from the LED (1).

The ends of a 1m long Ensign Beckford optical fibre, 0.5mm in diameter, numerical aperture of 0.65, were ground to a mirror finish using diamond paste on glass paste. One end of the fibre was mounted in an X
35 Y Z holder in front of a 20x microscope objective. The diodes (LED (1) and detector diodes (7) and (9)) were connected to a custom made driver/amplifier circuit as

- 22 -

shown in Figure 2. The circuit comprises a sender (10), current amplifiers (11) and (12), multiplexers (13) and (14), integrators (15) and (16) and an analog divider (17). The driver circuit was set to drive the LED (1) at 238Hz and the signals from the detector diodes (7) and (9) were switched between ground and the storage capacitors (integrator with a time constant of 1 second) synchronised with the driver signal. The two integrated signals correspond to background-corrected fluorescent signal and background corrected excitation light level (LED intensity). The former divided by the latter was supported by an analog divider as shown in Figure 2. For test purposes, the distal end of the fibre (6) was dipped into dilute solutions of rhodamine and the optics were adjusted for maximum signal from the analog divider.

Example 5

A fluorescence resonance energy transfer (FRET) glucose assay according to Meadows and Schultz (Talanta, 35, 145-150, 1988) was set up using concanavalin A-rhodamine (Cat No: C-860) and dextran-FITC (Cat No: D 1821, both from Molecular Probes Inc, Oregon, USA). The principle of the assay is fluorescence resonance energy transfer between the two fluorophores when they are in close proximity. In the presence of glucose the resonance energy transfer is inhibited and the fluorescent signal from FITC (fluorescein) increases. Thus increasing fluorescein fluorescence correlates with increasing glucose. A disposable soft contact lens (Johnson & Johnson, Acuvue, etafilcon A) of diameter 14.2 mm and correction -2.25 was removed from its packaging, blotted to remove excess storage fluid, returned to the storage container and 200 µl of the FRET glucose assay sensor chemistry was pipetted onto the lens. After incubating for 2 hours the lens was briefly

- 23 -

washed with excess distilled water, blotted dry (the lens retained the red coloured sensor chemistry components), a measurement of fluorescence was taken with the fibre optic spectrometer described in Example 4 and then the treated lens was placed in the eye of a human subject. After 15 minutes equilibration in lachrymal fluid the lens was removed and the fluorescence intensity ratio was measured. An increase in fluorescence intensity ratio from 0.8 to 0.98 was obtained indicating that the fluorescence resonance energy transfer assay chemistry had responded to glucose.

Example 6

The procedure of Example 5 was repeated using the sub eyelid device described in Example 3. A rise in the fluorescence intensity ratio from 0.85 to 0.92 was observed using the fibre optic spectrometer when the device was removed from the eye after 15 minutes in place.

Example 7

A sensor device comprising the sub-eyelid device treated with the FRET glucose sensor chemistry was inserted under the lower eyelid as described in Example 3. Immediately upon placing the sensing device in position a reading of fluorescence intensity from the device was taken using the fibre optic spectrometer. In this case the end of the fibre optic was placed against the lower eyelid and a measurement was made through the eyelid tissue with the eye partly closed. The fibre optic spectrometer was able to report a reading of the expected intensity ratio of 0.8 through the thin tissue of the eyelid, thus indicating that sensing devices can be operated in situ when placed under the eyelid.

- 24 -

Example 8

The FRET glucose sensor chemistry components were placed in an empty dropper bottle and then 2 drops (estimated at 100 μ l each) were delivered to the eye of a human subject by pulling the lower eyelid outwards and placing the drops between the lid and the eye. After blinking several times to distribute the fluid around the eye socket excess fluid was removed using a tissue placed against the closed eye. The eye was then inspected by pulling back the lower eyelid and the faint red tint of the rhodamine dye could be observed distributed evenly over the inner surface of the eyelid and on the lower part of the outer surface of the eyeball. Then the distal end of the fibre optic from the fibre optic spectrometer was brought almost into contact with the inner surface of the lower eyelid (while still pulled back) and a fluorescence intensity ratio reading of 0.92 was obtained. The fibre optic was then withdrawn and the eyelid returned to its normal position. Then the fibre optic was brought into contact with the outside of the lower eyelid and a fluorescence reading was obtained from the FRET glucose sensor chemistry through the thin tissue of the eyelid. Again a fluorescence intensity ratio of 0.92 was obtained. The FRET glucose sensor chemistry reagents were then removed using a standard eyewash solution and eye bath (Optrex Ltd, Nottingham, UK). After washing, measurements with the fibre optic spectrometer on the inside of the lower eyelid and from the external surface of the lower eyelid indicated that all the fluorescein and rhodamine fluorescence had been removed from the eye socket.

These experimental procedures were repeated using the FRET glucose sensor chemistry components diluted 1:1 with a commercially available eye treatment

- 25 -

preparation, Hypromellose Eye Drops, Martindale
Pharmaceuticals, Romford, UK which contains 0.3%
hypromellose polymer that could be used to extend the
residence time of the sensor chemistry materials in
5 the eye socket. Fluorescence ratio measurements from
the surface of the lower eyelid and the outer surface
of the eyeball were identical to those obtained above,
indicating that the hypromellose polymer was not a
quencher of the fluorescence signal from fluorescein
10 and rhodamine and could be useful in retaining the
sensor chemistry in situ over extended periods.

- 26 -

Claims:

1. A device for the detection or quantitative measurement of an analyte in lachrymal fluid, the device being locatable on the surface of the eyeball in contact with lachrymal fluid and incorporating an assay for said analyte, the readout of which assay is a detectable or measurable optical signal which can be interrogated remotely using optical means.
2. A device according to claim 1 wherein said assay is incorporated throughout the structure of the device.
3. A device according to claim 1 wherein said assay is incorporated within a discrete zone or spot.
4. A device according to claim 1 wherein said assay is incorporated in a strip on the periphery of the device.
5. A device according to any one of the preceding claims which is a full-size contact lens.
6. A device according to any one of claims 1 to 4 which is of a size suitable to permit location behind the lower eyelid.
7. A device according to claim 5 which is optically correcting.
8. A device according to claim 1 or claim 2 which comprises a fluid matrix in which the reactive components of said assay are suspended.
9. A device according to claim 8 which comprises a suspension of microcapsules within which

- 27 -

the reactive components of the assay are encapsulated.

10. A device according to claim 8 which
comprises a suspension of microparticles within which
5 the reactive components of the assay are embedded.

11. A device according to any one of the
preceding claims wherein said assay is a binding
assay, the readout of which is a detectable or
10 measurable optical signal.

12. A device according to claim 11 wherein said
binding assay is a competitive binding assay, the
reactive components of which include an analyte
15 binding agent and an analyte analog.

13. A device according to claim 12 wherein said
analyte analog is labelled with a first chromophore
and said analyte binding agent is labelled with a
20 second chromophore, the emission spectrum of said
first chromophore overlapping with the absorption
spectrum of said second chromophore.

14. A device according to claim 12 or claim 13
25 wherein said analyte binding agent is an antibody or a
fragment thereof, a lectin, a hormone receptor, an
enzyme, a drug receptor, an aptamer, a nucleic acid, a
nucleic acid analogue with specific binding capability
or a molecularly-imprinted polymer.

30

15. A device according to claim 11 wherein said
binding assay is a sandwich binding assay, the
reactive components of which include two distinct
analyte binding agents which are capable of
35 simultaneously binding to separate binding sites on
the same analyte.

- 28 -

16. A device according to any one of claims 11 to 15 wherein said detectable or measurable optical signal is generated by fluorescence energy transfer, fluorescence polarisation, fluorescence quenching, phosphorescence, luminescence enhancement, luminescence quenching, diffraction or plasmon resonance.

17. An analytical system for the detection or quantitative measurement of an analyte in lachrymal fluid, which analytical system comprises a device according to any one of the preceding claims together with optical means for interrogating the said device.

18. A method of quantitatively measuring the concentration of an analyte in the lachrymal fluid of a patient, said method comprising the steps of;

(a) locating a device according to any one of claims 1 to 16 in contact with the eyeball of said patient;

(b) allowing the assay of said device to reach thermodynamic equilibrium;

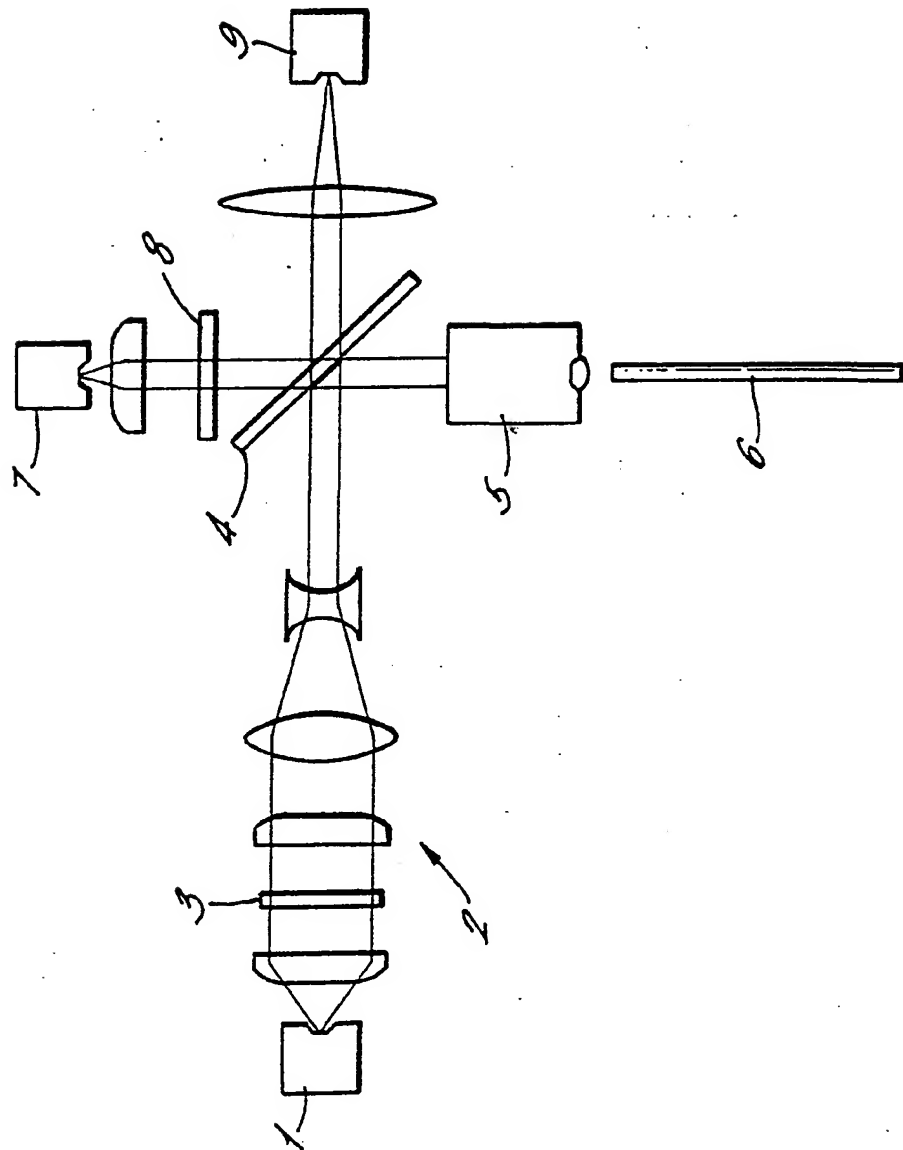
(c) interrogating the readout of said assay using optical means; and

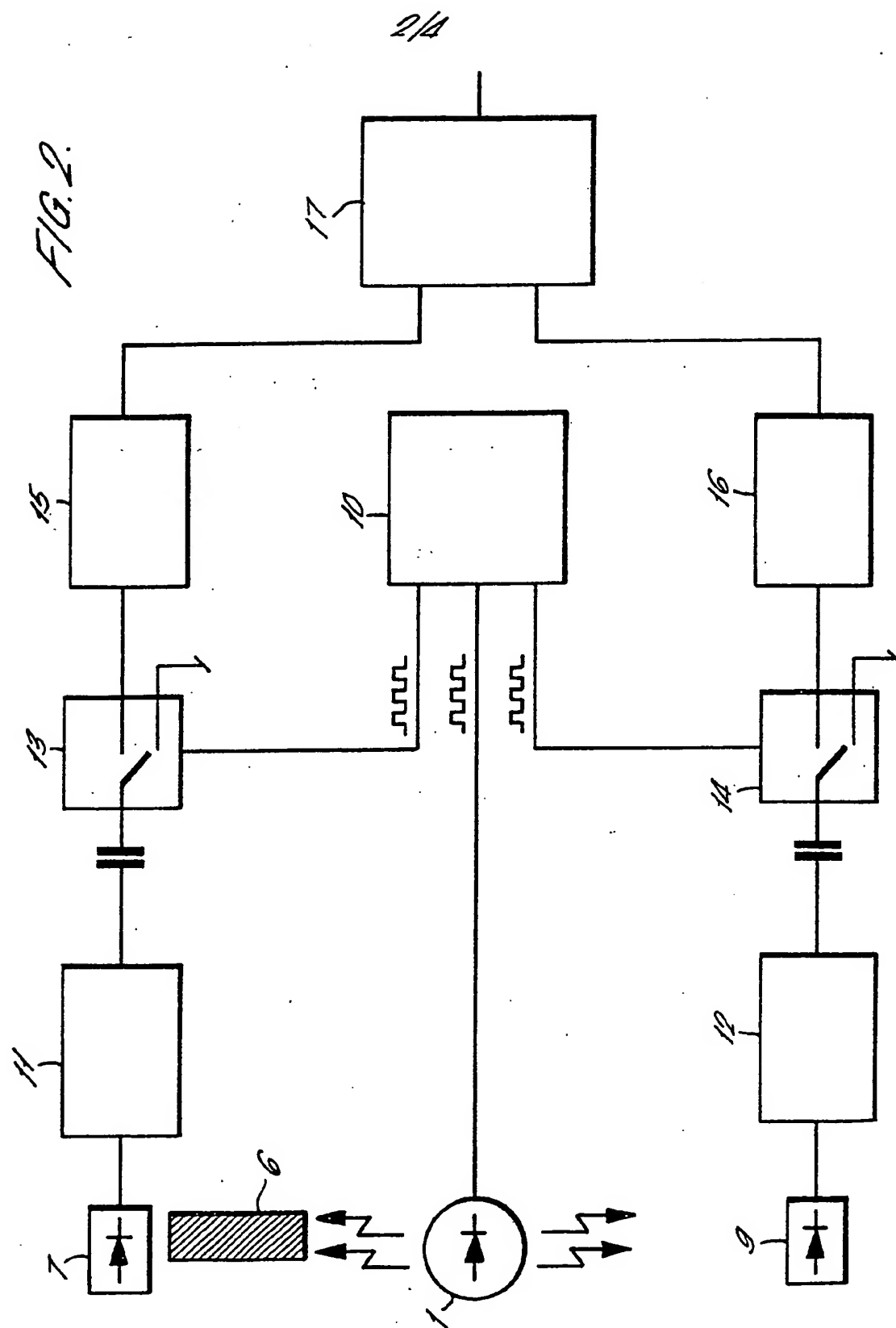
(d) relating the measurement obtaining in (c) to the concentration of analyte.

19. A method according to claim 18 wherein at least a part of the device is located behind the eyelid of the patient and in step (c) the readout of the assay is interrogated through the tissue of the eyelid.

1/4

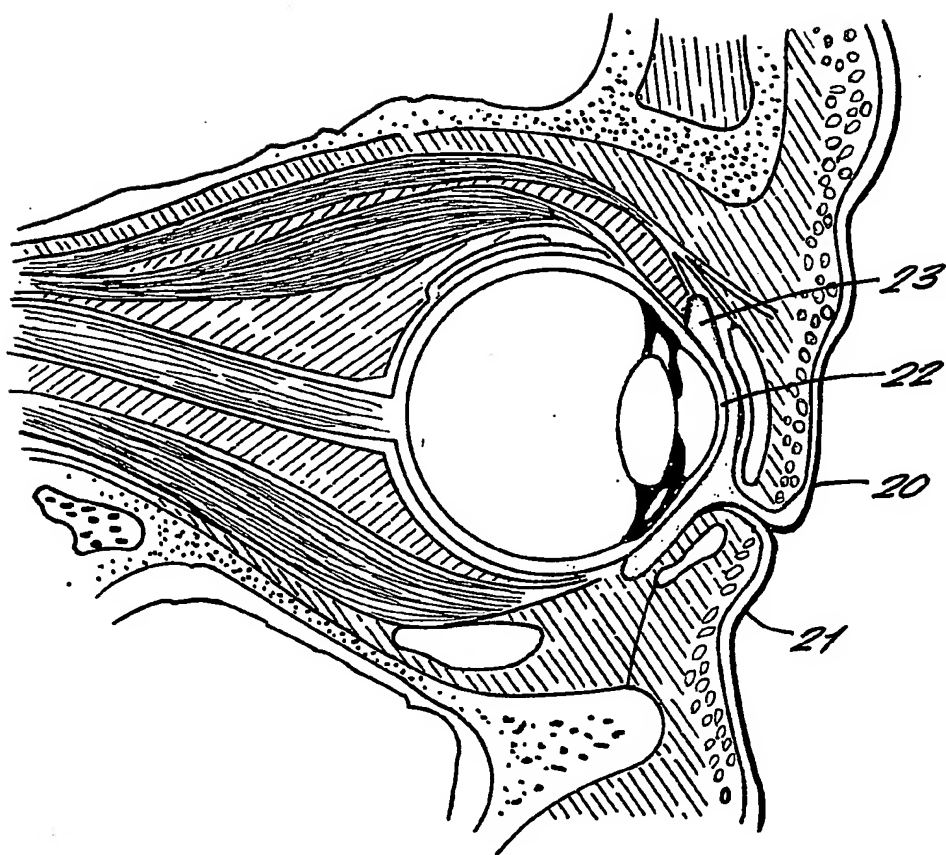
FIG. 1





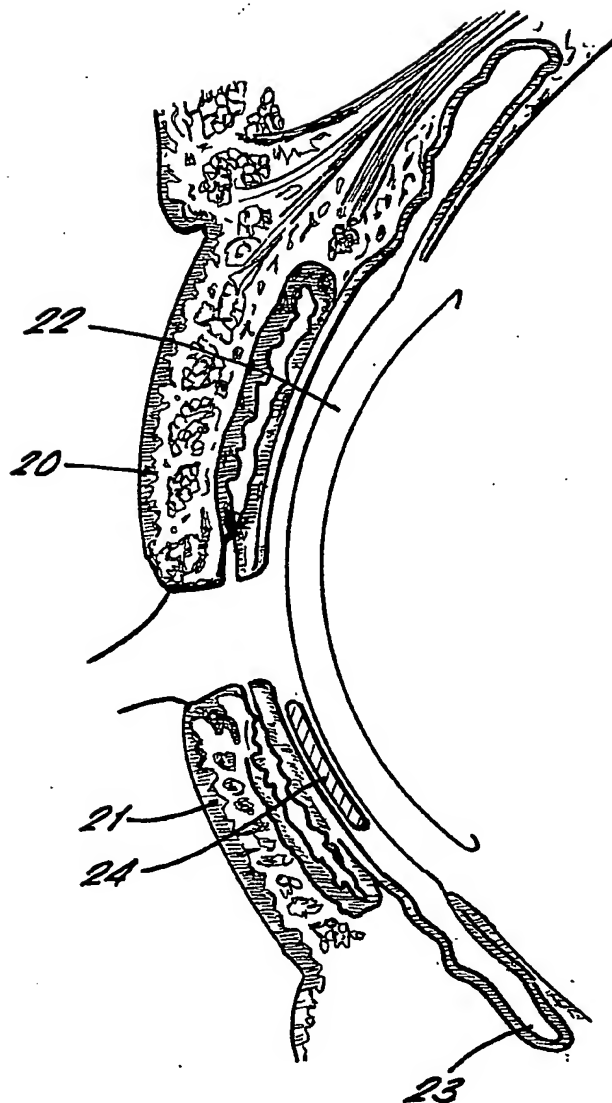
3/4

FIG. 3.



4/4

FIG. 4.



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 01/03078

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 4 597 392 A (LUEBBERS DIETRICH W ET AL) 1 July 1986 (1986-07-01) column 2, line 56 - column 3, line 46 ---	1,3,4,6, 17-19 5,11
X A	US 5 854 078 A (ASHER SANFORD A ET AL) 29 December 1998 (1998-12-29) column 10, line 3 - line 12 column 10, line 56 - line 65 column 14, line 26 - line 35 --- -/--	1-8,11, 12,14, 17,18 16,19

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

C document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

19 September 2001

Date of mailing of the international search report

08/10/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Knüpling, M

INTERNATIONAL SEARCH REPORT

In International Application No

PCT/GB 01/03078

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUSSELL R J ET AL: "A FLUORESCENCE-BASED GLUCOSE BIOSENSOR USING CONCAVALIN A AND DEXTRAN ENCAPSULATED IN A POLY(ETHYLENE GLYCOL) HYDROGEL" ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. COLUMBUS, US, vol. 71, no. 15, 1 August 1999 (1999-08-01), pages 3126-3132, XP000851425 ISSN: 0003-2700 abstract -----	1,8-14, 16,18
A	US 4 999 287 A (ALLEN MICHAEL P ET AL) 12 March 1991 (1991-03-12) column 4, line 42 - line 48 column 5, line 13 - line 22 -----	1,11,15, 18
A	MARCH W.F. ET AL: "Intraocular lens glucose sensor" DIABETES TECHNOLOGY & THERAPEUTICS, vol. 2, no. 1, 2000, pages 27-30, XP001028056 page 27-28, section "Materials and Methods" -----	1,2,8, 11-14, 16-18

INTERNATIONAL SEARCH REPORT

Int lional Application No

PCT/GB 01/03078

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4597392	A	01-07-1986	DE 3313047 A1	18-10-1984
			EP 0121831 A2	17-10-1984
			JP 1745154 C	25-03-1993
			JP 4033456 B	03-06-1992
			JP 59207135 A	24-11-1984
US 5854078	A	29-12-1998	US 5898004 A	27-04-1999
			AU 6693598 A	12-10-1998
			EP 0986750 A1	22-03-2000
			WO 9841859 A1	24-09-1998
			US 6187599 B1	13-02-2001
			AU 717930 B2	06-04-2000
			AU 5093698 A	29-05-1998
			EP 0951348 A1	27-10-1999
			JP 2001505236 T	17-04-2001
US 4999287	A	12-03-1991	WO 9819787 A1	14-05-1998
			AT 115631 T	15-12-1994
			AU 626853 B2	13-08-1992
			AU 3384789 A	23-11-1989
			DE 68919902 D1	26-01-1995
			DE 68919902 T2	11-05-1995
			EP 0342447 A2	23-11-1989
			JP 2138961 A	28-05-1990
			US 5423989 A	13-06-1995
			US 4987085 A	22-01-1991
			US 5215886 A	01-06-1993

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.